AS

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 203 676**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **86301223.3**

(22) Date of filing: **20.02.86**

(51) Int. Cl.⁴: **A 61 K 39/12**, A 61 K 37/02, A 61 K 39/245

(30) Priority: **19.04.85 US 725087**

(43) Date of publication of application: **03.12.86 Bulletin 86/49**

(84) Designated Contracting States: **AT BE CH DE FR GB IT LI LU NL SE**

(71) Applicant: **THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, 36th & Spruce Streets, Philadelpia, PA 19104 (US)**

(72) Inventor: **Heber-Katz, Ellen, 2300 Walnut Street, Philadelphia Pennsylvania 19103 (US)**

(74) Representative: **Newby, John Ross et al, J.Y. & G.W. Johnson Furnival House 14/18 High Holborn, London WC1V 6DE (GB)**

(54) **Vaccine for generating an immunogenic T cell response protective against a virus.**

(57) A vaccine for generating an immunogenic T cell response protective against a virus, such as a herpes virus, comprising an immunologically effective amount of (1) a peptide-fatty acid conjugate, the peptide having an amino acid sequence corresponding to the sequence of a fragment of a glycoprotein of the virus which produces a T cell response, or a synthetic replica of such fragment, (2) a liposome composition comprising a mixture of phosphatidyl choline, cholesterol and lysophosphatidyl choline, and (3) complete Freund's adjuvant.

EP 0 203 676 A2

VACCINE FOR GENERATING AN IMMUNOGENIC T CELL RESPONSE PROTECTIVE AGAINST A VIRUS

Background of the Invention

1. Field of the Invention

This invention relates to preparation of a vaccine for generating an immunogenic T cell response protective against a virus. Although the invention is described with particularity with respect to a vaccine which provides significant protection for an extended period of time against a large dose of herpes virus, it is to be understood that according to the invention vaccines may be prepared which offer protection against a number of other viruses such as rabies, influenza, HTLV-III (AIDS), retroviruses and oncogenic viruses.

Herpes viruses are widely spread in nature and natural hosts include foul and animals, including man. Man is the natural host for herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2, respectively), varicella/zoster, cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Herpes simplex viruses are known to cause many human diseases such as cold sores, encephalitis, eye infections, and genital infections, and HSV-2 has also been linked to cervical carcinoma. Clinical illness caused by herpes viruses presents a significent health problem and HSV-2 has produced major sociological consequences. At the present time no effective preventative measures are available.

There are experimental data, however, which indicate that immunization with a herpes virus or surface components of the virus can be protective. One

such component, the virus envelope glycoprotein D molecule (gD), which is 59,000 MW, has been shown to be protective (Long, et al, Infect. and Immunity. 37: 761-764 (1984)). It has also been shown that antibody to this molecule can neutralize the virus and, when passively injected into animals, can be protective (Balanchandran et al, Infect. and Immunity, 37: 1132-1137 (1982); Dix et al., Infect. and Immun., 34: 192-199 (1981); Kapoor et al., J. Gen. Virol., 60: 225-233 (1982)). In all protocols for immunization against herpes virus thus published, high antibody titers have been reported. Therefore, it has been concluded generally that high antibody titers are extremely important in providing protection. Other studies indicate that such conclusion pertains to other virus infections as well.

It is known, however, that (1) recurrent herpes infections often occur in the presence of high antibody levels in the serum of patients and (2) individuals who have been infected previously with HSV-1 and are synthesizing antibody which crossreacts with both HSV-1 and HSV-2, nevertheless, contract HSV-2. More importantly, there have been studies showing not only the inability of antibody to protect, but that antibody can actually interfere with a protective host immune response (Wilson, et al., J. Immunology 132: 1522-1528 (1984); Babiuk et al., J. Microbiology, 25: 267 (1979)).

2. Objects of the Invention

It is an object of the invention to produce vaccines that offer significant protection for a long

period of time against a large dose of virus, especially a herpes virus.

It is a further object to achieve such protection by a limited number of immunizations, in many instances a single immunization.

Another object of the invention is the development of an anti-HSV vaccine which utilizes a T cell response.

These and other objects of the invention will become further apparent from the detailed description of the invention, the appended claims, and the drawings in which

Figure 1 is a plot of the percentage of normal mice vs. time (days) comparing the protection against lethal infection by HSV-2 provided by the vaccine of the present invention as compared to certain control inoculants;

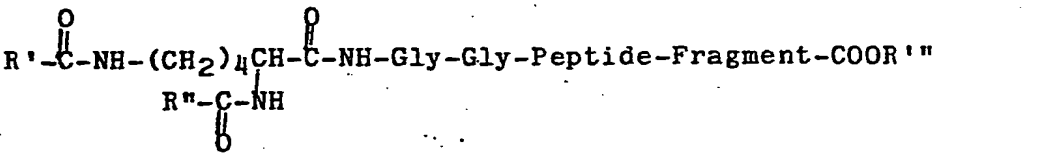
Figures 2a and 2b show the antibody binding activity induced by the vaccine of the invention in relation to certain control inoculants, and

Figure 3 is similar to Fig. 1, but using a reduced concentration of herpes virus ($4LD_{50}$ vs. $10LD_{50}$) in the challenge.

Summary of the Invention

The vaccines of the present invention have been found to generate a T cell response protective against a virus, such as a herpes virus, and comprise an immunologically effective amount of (1) a peptide-fatty acid conjugate, the peptide having an amino acid sequence corresponding to the sequence of a fragment of a glycoprotein or protein of the virus which produces a T cell response or a synthetic replica of the

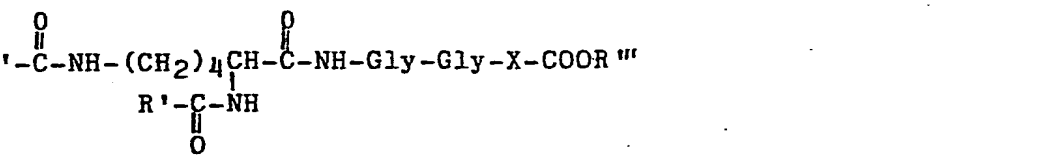
glycoprotein or protein fragment, the conjugate having the formula



where R' and R" are alkyl groups containing from 5 to 30, preferably from 10 to 20 carbon atoms, and R'" is selected from the group consisting of hydrogen and at least one amino acid residue; (2) a liposome composition comprising a mixture of phosphatidyl choline, cholesterol and lysophosphatidyl choline, and (3) adjuvant.

As used in the specification and appended claims, the expression "T cell response" refers to the ability of T cells to respond to antigen by production of lymphokines and/or molecules involved in effector functions, other than help for B cells in production of antibody, after antigen stimulation.

A preferred vaccine is an anti-HSV-1 or HSV-2 vaccine in which the immunologically active peptide-fatty acid conjugate has an amino acid sequence corresponding to a fragment of herpes simplex virus type 1 envelope glycoprotein gD-1 or herpes simplex virus type 2 envelope glycoprotein gD-2 which produces a T cell response or a synthetic replica of such glycoprotein fragment, the conjugate having the formula



where R', R" and R'" are as stated above, and X is an amino acid sequence corresponding to that of a fragment

of a herpes virus envelope glycoprotein, components (2) and (3) of the vaccine being as previously indicated. In a particularly preferred vaccine of the invention X has the amino acid sequence

-Ser-Leu-Lys-Met-Ala-Asp-Pro-
Asn-Arg-Phe-Arg-
Gly-Lys-Asn-Leu-Pro-

R' and R" are alkyl groups containing 15 carbon atoms, R'" is a cysteine residue and the adjuvant is alum.

Such peptide fatty acid conjugates may be prepared for example by adding a spacer of Gly-Gly-Lys to an N-terminal region of a virus glycoprotein which produces a T cell response and then adding two fatty acid side chains to the amines of the N-terminal lysine, the coupling of the fatty acid moieties being carried out by the symmetric anhydride method (Hopp, Molecular Immunology, 21: 13-16 (1984)).

According to a preferred form of the invention, the Gly-Gly-Lys spacer is added to an N-terminal region or fragment of the glycoprotein D molecule of HSV-1 or HSV-2 which produce a T cell response, following which a palmitic acid side chain is linked to each of the alpha and epsilon amino groups of the terminal lysine.

The N-terminal region of either gD-1 or gD-2 may comprise a synthetic peptide with sequence homology manually synthesized using Merrifield solid phase methods (Merrifield, J. Am. Chem. Soc. 85: 2194 (1963); Stewart et al, (ed), Solid phase peptide synthesis, W.H. Freeman and Co., San Francisco, CA (1969)).

The resulting products are useful as anti-virus vaccines, particularly anti-HSV-1 and anti-HSV-2 vaccines. The process is applicable to

viruses generally and particularly to other members of the herpes virus group.

As stated, it was discovered that the vaccines of the invention achieve significant protection for a protracted period of time against a large dose of virus by a single injection. Considering the fact that prior researchers generally concluded that high antibody titers are extremely important in providing protection against HSV-1 and HSV-2 infection, it was surprising that a protective immune response against HSV infection need not stimulate an antibody response. Rather it was discovered that with vaccines of this invention a T cell proliferative response in the absence of an antibody response leads to effective long term protection.

Detailed Description of the Invention

The invention is described hereinafter in detail with respect to preparation and testing for immunogenicity of an anti-HSV-1 or HSV-2 vaccine comprising a synthetic peptide-fatty acid conjugate, the synthetic peptide having sequence homology with an N-terminal region or fragment of envelope glycoprotein D (gD) of either HSV-1 or HSV-2 which produces a T cell response. However, as previously noted the peptide portion of the conjugate may comprise a fragment of a protein or glycoprotein, usually a surface glycoprotein of a virus, other than a herpes virus.

Considering the constituent parts of the vaccine, the HSV specific component of the peptide-fatty acid conjugate comprises an N-terminal fragment of the surface glycoprotein D (gD), i.e. an amino acid peptide chain which produces a T cell response. The peptide may be the 23 amino acid peptide chain, the sequence of

which was deduced from a terminal sequence of the gD molecule, a protein component of HSV-2 (Watson, Gene 158: 303 (1983)). The entire 23 amino acid sequence of the N-terminal fragment of HSV-2 gD is

(1) (2) (3) (4) (5) (6) (7)
NH_2-Lys-Tyr-Ala-Leu-Ala-Asp-Pro-

(8) (9)(10)(11)(12)(13)(14)(15)
Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-

(16)(17)(18)(19)(20)(21)(22)
Arg-Phe-Arg-Gly-Lys-Asn-Leu-

(23)
Pro-COOH

The 23 amino acid sequence of the N-terminal fragment of HSV-1 gD differs from that of HSV-2 gD in that alanine is at position 7 and aspartic acid is at position 21 (Dietzschold et al., J. of Viro., 52, No.2: 431-435 (1984)).

Rather than using the entire 23 amino acid N-termial protein of HSV-2 gD or HSV-1 gD, certain subunits thereof may be used provided they give a T cell response. A typical subunit of HSV-2 gD is

(8) (9)(10)(11)(12)(13)(14)
-Ser-Leu-Lys-Met-Ala-Asp-Pro-

(15)(16)(17)(18)
-Asn-Arg-Phe-Arg-

(19)(20)(21)(22)(23)
-Gly-Lys-Asn-Leu-Pro-

As previously stated the synthetic replicas of the above-noted N-terminal fragments may be prepared using solid phase methods (see Merrifield, J. Am. Chem. Soc. 65: 2149 (1963); Stewart et al (ed), Solid phase peptide synthesis, W.H. Freeman and Co., San Francisco (1969)).

There is added to the N-terminus of the peptide fragments a spacer of Gly-Gly-Lys$(NH_2)_2$. Such addition can be accomplished as part of the synthesis of the N-terminal peptide fragment. Preferably, cysteine is also added at the C-terminus for linkage to other "carrier" molecules, which are generally relatively large proteins.

There are then coupled to the alpha and epsilon amines of the lysine terminus saturated fatty acids generally having from about 11 to about 21 carbon atoms, examples of which are palmitic, stearic and oleic acids. The procedure by which conjugates of this general type are formed is well known (Hopp, Molecular Immunology. 21: 13-16 (1984)).

The peptide-fatty acid conjugate, prepared as above, is mixed with a liposome composition comprising a mixture of three lipids, namely phosphatidyl choline, cholesterol and lysophosphatidyl choline, following the method of Thibodeau (see Thibodeau et al, "Genetic variation among influenza viruses", Acad. Press, N.Y., London (1981) p 587). The weight proportion of the three constituents of which the liposome is formed may vary considerably. Preferably, the proportion by weight of the three lipids is 16:2:1, respectively.

The peptide-fatty acid conjugate-containing liposome preparation is then mixed with an adjuvant, e.g. alum or complete Freund's adjuvant. Generally a weight ratio of 1:1 is used when the adjuvant is CFA and results in an emulsion which is available for use as a vaccine. In the case of alum, from about 4 to about 16 parts, preferably about 8 parts by weight per part of peptide (in the absence of spacer and fatty acid) may generally be used.

The vaccine is administered in a dosage range of from about 100 to 300 μg, preferably about 150 μg, based on the weight of peptide per se (in the absence of spacer and fatty acid), in order to obtain the desired immunogenic T cell response protective against HSV-1 and HSV-2 virus. Usually the vaccine may be administered in a single dose and protection against a large dose of herpes virus is provided. However, a series of doses at intervals of several weeks or months followed, if necessary, by a booster dose at an interval of serval months to several years may be administered if necessary. So used, the vaccine will produce in laboratory animals a T cell response protective against a herpes virus.

The following examples further illustrate the present invention without, however, limiting the same thereto.

Example 1

A peptide having the sequence homology of the 23 amino acid peptide chain, the sequence of which was deduced from a N-terminal gD molecule of HSV-2, having a spacer of Gly-Gly-Lys added to the N-terminus and cysteine added to the C-terminus, and having the following formula was prepared by Merrifield solid phase methods (Merrifield 1963; Stewart et al, 1969, supra)

$(NH_2)_2$Lys-Gly-Gly)*-Lys-Tyr-Ala-Leu-
Ala-Asp-Pro-Ser-Leu-Lys-Met-
Ala-Asp-Pro-Asn-Arg-Phe-Arg-
Gly-Lys-Asn-Leu-Pro-(Cys)**COOH

* A spacer group added to the peptide and to which palmitic acid side chains are added to the Lys amino groups.

** A cysteine amino acid used for linkage to other carrier molecules.

More specifically the peptide molecule having the amino acid sequence immediately above, and sometimes referred to herein as "1-23(2)", was synthesized as follows:

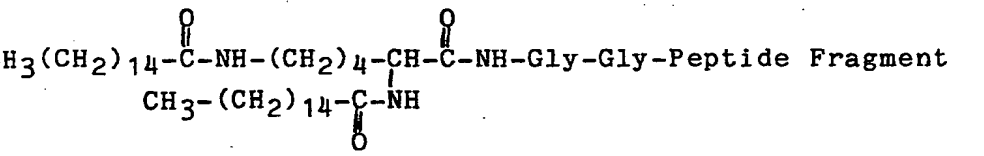
All N-tert-butoxycarbonyl (BOC) amino acids were purchased from Sigma Chemical Co., BOC-Cys-O-Resin, L-t-Amyloxycarbonyl-N-Tosyl-L-Arginine, and L-BOC-O-Benzyl-L-Serine were purchased from Peninsula Laboratories, Inc. Peptides were manually synthesized using Merrifield solid phase methods (Merrifield, 1963; Stewart, 1969, supra) with the following modifications.

(1) A series of three washes, the first with methylene chloride, the second with absolute ethanol and the third with methylene chloride was used instead of dioxane and chloroform before and after deprotection of N-t-BOC amino acid groups; (2) N-t-BOC amino acids were deprotected with 25% trifluoroacetic acid in methylene chloride; (3) completeness of the deprotection and coupling reactions was monitored using the color tests described by Kaiser et al., Annal. Biochemistry, 34: 595-598 (1970). After synthesis the resin was dried, and 50 equivalents of thioanisole were added. The side protection groups were removed and the peptide was cleaved from the resin with anyhydrous hydrogen fluoride. After removal of the anyhydrous hydrogen

fluoride, the peptide resin mixture was extensively washed with ethyl acetate and ether to remove the thioanisole. The cleaved peptide was extracted with 1.5% NH_4CO_3 and lyophilized. The amino acid sequence of the peptide was verified by automated Edman degradation as described by Hunkapillar and Hood (Biochemistry 17: 2124-2133 (1978)).

Example 2

To add palmitic acid side chains, the N-terminal lysine was coupled as the bis-t-butyloxycarbonyl derivative, then deprotected using trifluoroacetic acid. The palmitic acid moieties were coupled by the symmetric anhydride method (Hopp, 1984, supra). Thus, the molecule is:



Example 3

The fatty acid-peptide conjugate of Example 2 was then mixed with a liposome comprising 3 lipids as follows (see Thibodeau et al, (1981)):

Phosphatidyl choline, cholesterol, and lysolecithin were each dissolved in MeOH/chloroform (1:3) and then mixed in the ratio of 16:2:1, respectively. This mixture was then blown down with N_2 rotating the vial in warm H_2O to get an even film over the entire vial. Five mg. of the peptide-palmitic acid conjugate was dissolved in 2 ml of a 1% octylglycoside in phosphate buffered saline solution (PBS). Ten mg. of the lipid mixture was then added to this peptide

solution. Dialysis was carried out against PBS using a 3500 dalton cutoff Spectropore dialysis mebrane for 24 hours. The liposomes were sonicated for 5 minutes.

The peptide-containing liposome preparation was then mixed with complete Fruend's adjuvant (CFA) in a ratio of 1:1 to form a vaccine emulsion.

Example 4

Testing of the vaccine.

The first experiment was done with a single injection of vaccine into the two hind footpads of a group of six Balb/c mice. The volume given was approximately 0.2 ml/animal or 10 μg/g body weight. In addition, as controls Balb/c mice (6 mice/group) were immunized with 1-23(2) peptide in CFA, CFA alone, and UV-inactivated HSV-1 in CFA, as follows:

(1) 1-23(2) at 100 μg/animal;

(2) UV-inactivated HSV-1 at 10 PFU/animal;

(3) Vaccine of Example 3 (1-23(2)-palmitic acid-liposome, CFA) at approximately 150 μg peptide/animal;

(4) CFA alone.

Six and 1/2 months after this single immunization, the mice were challenged with a lethal dose of HSV-2 (a 10 LD_{50} dose of strain 186 grown in BHK cells which are mouse fibroblasts) in both hind footpads and the animals were examined for the next 30 days for paralysis and death. The results are plotted in Figure 1. It can be seen that by day 8, many of the animals were symptomatic. Only two groups, the HSV-1-primed (-◆-◆-) and the 1-23(2) palmitic acid-liposome-CFA-primed (-□-□-) animals, appeared normal. On day 18, when all of the control animals had died (CFA-◇-◇-; 1-23(2) (-■-■-), the HSV-1 and

1-23(2)-palmitic acid-liposome, CFA vaccine immunized animals survived. At day 120, 33% of both of those groups were normal.

Example 5

To determine the mechanism of protection, the antibody response (humoral response in terms of antibody which can bind the peptide to virus and which can neutralize the activity of virus infectivity in an in vitro assay) was studied. T cells responses, by measuring the ability of T cells to respond to antigen by their production of lymphokines after antigen simulation were also determined. First, since it is known that neutralizing antibody can protect animals from an HSV infection (Balachandran, 1982 supra; Dix, et al, Infec. and Immun., 34: 192-199 (1981) Kapoor et al, J. Gen. Virol., 60: 225-233 (1982)), an effort was made to determine if this was the cause of the protection seen.

Animals were bled 1 week after HSV-2 challenge since specific antibody which had already been induced by previous exposure to antigen (peptide) should at that time have been high, and serum was taken from animals and frozen until time of assay. The results obtained as seth forth in Table 1, below.

TABLE 1

Neutralization Titers of Anti-HSV Antibody

Pooled Serum* From Each Group	Neutralizing Antibody for HSV-1	HSV-2
1. Control	12**	6
2. HSV-1	389	97
3. 1-23(2)	6	8
4. Vaccine of Example 3 (1-23(2)-liposome CFA)	5	5

* Animals were challenged with HSV-2 in the foodpads 6 and 1/2 months after a single immunization of antigen in CFA. Bleedings were done 1 week after challenge with HSV-2. 100 PFU of virus in 25 microliters were added to serum antibody in the same volume with the serum being diluted in two fold dilutions. This mixture in 96 well Costar plates was incubated for 1 hour at 37°C and BHK cells were added at a concentration of 5×10^4 cells/ml in 50 microliters. Three to four days later, the cells were stained with crystal violet dissolved in 10% buffered formalin.

** Titer=1/dilution of sample x 2^x; x=well number

As shown in Table 1, neutralizing antibody titers could explain the protection in the HSV-1 group, but could not explain the protection seen with the group immunized with the vaccine of Example 3 (1-23(2)-liposome CFA).

Example 6

This example pertains to detection of anti-viral binding activity and should be considered in connection with Figures 2(a) and 2(b). Balb/c mice were challenged with HSV-2 in the hind footpads 6 and 1/2 months after single immunization with the vaccine of Example 3. Bleedings were done 1 week after challenge with HSV-2. A radioimmunoassay was carried out using HSV-1 (-○-○-) and HSV-2 (-△-△-) infected and uninfected (-■-■-) BHK lysates, and 1-23(H) peptides (-●-●-) and

phosphate buffered saline alone (-▼-▼-) as immunoadsorbants. The assay was done by (a) preincubation of vinyl 96 well culture plates with the various immunoadsorbants in saline overnite at 4°C; (b) coating with 50% Fetal Calf Serum; (c) incubation of the Balb/c antisera for 3 hours at room temperature, and (d) labeling with 5000 CPM of ^{125}I-rabbit Fab anti-mouse Ig. Figures 2(a) and 2(b) show the amount of radioactively labeled anti-mouse antibody bound versus the dilution of the antiserum tested. The highest concentration shown is considered the nonspecific binding region. As shown by the figures, anti-peptide binding activity at the highest concentration tested could be detected when the animals have been immunized with 1-23(2)-lipsome (A) and 1-23-(2) (C), but not with HSV-1 (B) or CFA (D). The nature of the binding activity is unclear since it is detected in the non-specific part of the titration curve. However, it is clear that antibody to the virus was detected only in animals immunized with HSV-1 (B).

In view of these experiments it was concluded that the vaccine of the invention induces no detectable antibody which can bind the virus.

Example 7

T cells from animals immunized with the vaccine of Example 3 were obtained from the lymph nodes, purified on nylon wool columns (Julius, et al, Europ. J. Immunol. 3: 645 (1977)), and then tested for responsiveness in vitro by T cell proliferation measured through the incorporation of ^{3}H-thymidine into DNA after three days in culture (Corradin, et al., J. Immunol. 119: 1046 (1977)). The results appear in Table 2.

TABLE 2

T cells alone	7,300 CPM
T cells + 1-23(2) peptide 50 μg/ml	30,400 CPM
T cells + HSV-1 (10^6 PFU/ml)	20,300 CPM

The data in Table 2 show that T cells from animals vaccinated respond to the peptide and also cross-react with the virus. The T cell proliferation test in vitro has generally been accepted as indicative of the presence of an antigen specific T cell response as correlating with T cell effector function. T cells induced by the peptide-containing vaccine are responsive not only to the specific peptide but also to a corresponding exiologic agent of the herpes virus infection, i.e. HSV-1.

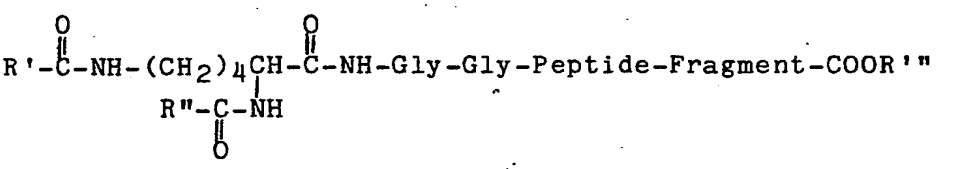
Example 8

A further experiment was carried out in the same manner as Example 4. Animals were immunized in the hind footpads with the following: (a) 1-23(2)-palmitic acid-liposomes in CFA (13 animals) (-□-□-); (b) UV-inactivated HSV-1 in CFA (5 animals) (-□-□-); (c) CFA alone (10 animals) (-◇-◇-); (d) liposomein in CFA (11 animals) (-◆-◆-); and (e) 1-23(2)-palmitic acid-liposome in saline (5 animals) (-■-■-). The animals were then challenged 3 and 1/2 months after immunization with a 4 LD 50 of HSV-2(186). The results are presented in Figure 3.

The published articles identified in the foregoing specification are incorporated by reference herein in their entirety.

Claims

1. A vaccine for generating an immunogenic T cell response protective against a virus, said vaccine comprising an immunologically effective amount of (1) a peptide-fatty acid conjugate, said peptide having an amino acid sequence corresponding to the sequence of a fragment of a protein or glycoprotein of said virus which produces a T cell response or a synthetic replica of said fragment, said conjugate having the formula



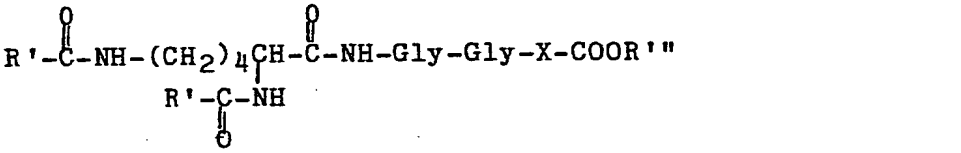
where R' and R" are alkyl groups containing from 5 to 30 carbon atoms, and R'" is selected from the group consisting of hydrogen and at least one amino acid residue; (2) a liposome composition comprising a mixture of phosphatidyl choline, cholesterol and lysophosphatidyl choline, and (3) an adjuvant.

2. A vaccine according to claim 1 in which each of R' and R" is an alkyl group containing from 10 to 20 carbon atoms, R'" is a cysteine residue, and the adjuvant is selected from the group consisting of alum and complete Freund's adjuvant.

3. A vaccine according to claim 1 in which each R' and R" is an alkyl group containing 15 carbon atoms, R'" is a cysteine residue, and the adjuvant is alum.

4. A vaccine according to claim 3 in which said phosphatidyl choline, cholesterol and lysophosphatidyl choline are present in the proportions by weight of 16:2:1, respectively.

5. A vaccine for generating an immunogenic T cell response protective against a herpes simplex virus type 1 or type 2 disease state, said vaccine comprising a peptide-fatty acid conjugate, said peptide having an amino acid sequence corresponding to a fragment of herpes simplex virus type 1 envelope glycoprotein gD-1 or herpes simplex virus type 2 envelope glycoprotein gD-2 which produces a T cell response or a synthetic replica of said fragment, said conjugate having the formula



where R' and R" are alkyl groups containing 5 to 30 carbon atoms, and R'" is selected from the group consisting of hydrogen and at least one amino acid residue, and X is an amino acid sequence corresponding to that of a fragment of herpes virus envelope glycoprotein, (2) a liposome composition comprising a mixture of phosphatidyl choline, cholesterol and lysophosphatidyl choline, and (3) an adjuvant.

6. A vaccine according to claim 5 in which each of R' and R" is an alkyl group containing from 10 to 20 carbon atoms, R'" is a cysteine residue, and the adjuvant is selected from the group consisting of alum and complete Freund's adjuvant.

7. A vaccine according to claim 5 in which each of R' and R" is an alkyl group containing 15 carbon atoms, R'" is a cysteine residue, and the adjuvant is alum.

8. A vaccine according to claim 5 in which said phosphatidyl choline, cholesterol and lysophosphatidyl choline are present in the proportions by weight of 16:2:1, respectively.

9. A vaccine according to claim 5 in which X is

-Ser-Leu-Lys-Met-Ala-Asp-Pro-
Asn-Arg-Phe-Arg-Gly-Lys-Asn-
Leu-Pro-

10. A vaccine according to claim 5 in which X is

-Lys-Tyr-Ala-Leu-Ala-Asp-Pro-
Ser-Leu-Lys-Met-Ala-Asp-Pro-
Asn-Arg-Phe-Arg-Gly-Lys-Asn-
Leu-Pro-

each of R'and R" is an alkyl group containing 15 carbon atoms, R'" is a residue of cysteine, and the adjuvant is alum.

11. A vaccine according to claim 5 in which X is

-Ser-Leu-Lys-Met-Ala-Asp-Pro
Asn-Arg-Phe-Arg-Gly-Lys-
Asp-Leu-Pro-

12. A vaccine according to claim 5 in which X is

Lys-Tyr-Ala-Leu-Ala-Asp-Ala-
Ser-Leu-Lys-Met-Ala-Asp-Pro-Asn-
Arg-Phe-Arg-Gly-Lys-Asp-Leu-Pro

J Y & G W JOHNSON

0203676

Chartered Patent Agents
European Patent Attorneys

John R Newby MS(USA) BSc AKC
Stephen G Hale MA(Cantab)
Martin J Nawby BSc

Raymond L Newby BSc C Eng. MIMechE - Consultant

Assisted by:
Colin M Dibble MITMA - Trade Marks
Edward Cook - Accounts

NEW TELECOPIER NUMBER
(II & III) 01-831 9528

FURNIVAL HOUSE
14-18 HIGH HOLBORN
LONDON WC1V 6DE

Telephone: 01-405 0356
01-405 2219
Telex: 27183 JYJOHN G
Cables: JYJOHN LONDONWC1
Telecopier: (II & III) 01-405 7411
- Quote "JYJOHN"

European Patent Office,
P.B. 5818,
Patentlaan 2,
2280 HV Rijswijk (ZH),
Netherland.

Our Ref SGH/JEG

Your Ref

Date 27th August 1986

Dear Sirs,

Application No. 86301223.3 -
The Wistar Institute of Anatomy and Biology

Under the provisions of Rule 88 EPC I request correction of errors in the formulae at page 4 line 25 and in claim 5. In each case the lower of the two groups R' should be R" in agreement with the formulae at line 3 of page 4 and in claim 1. I submit that both the error and its correction are obvious in the context.

Yours truly,

S.G. HALE

P.S. I enclose one copy of original pages 4 and 18 showing the corrections in red and three copies of the corrected pages 4 and 18.

0203676

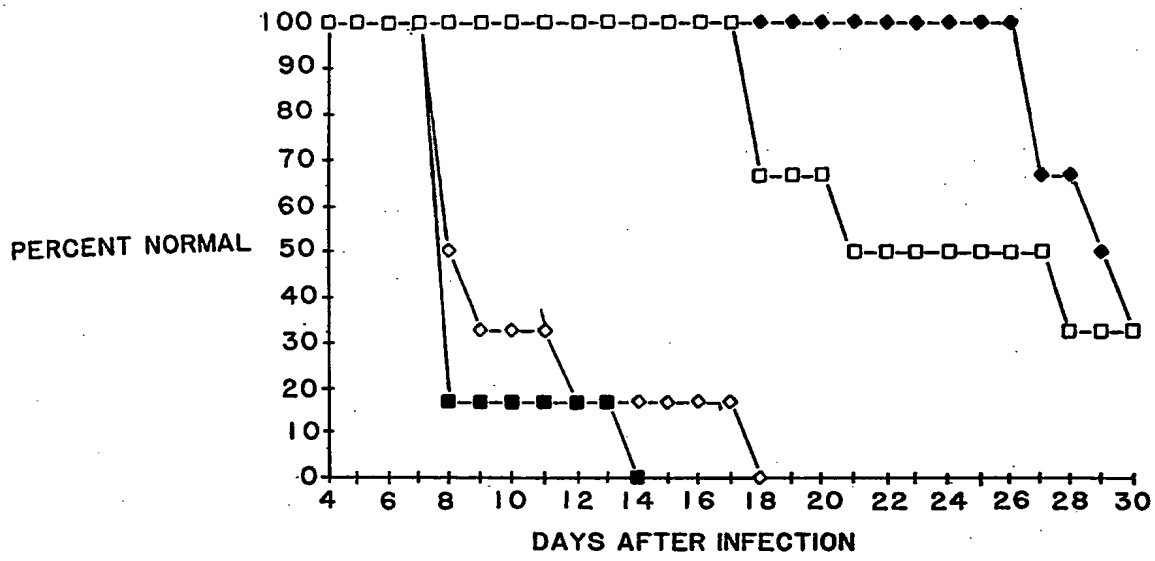


FIG. 1

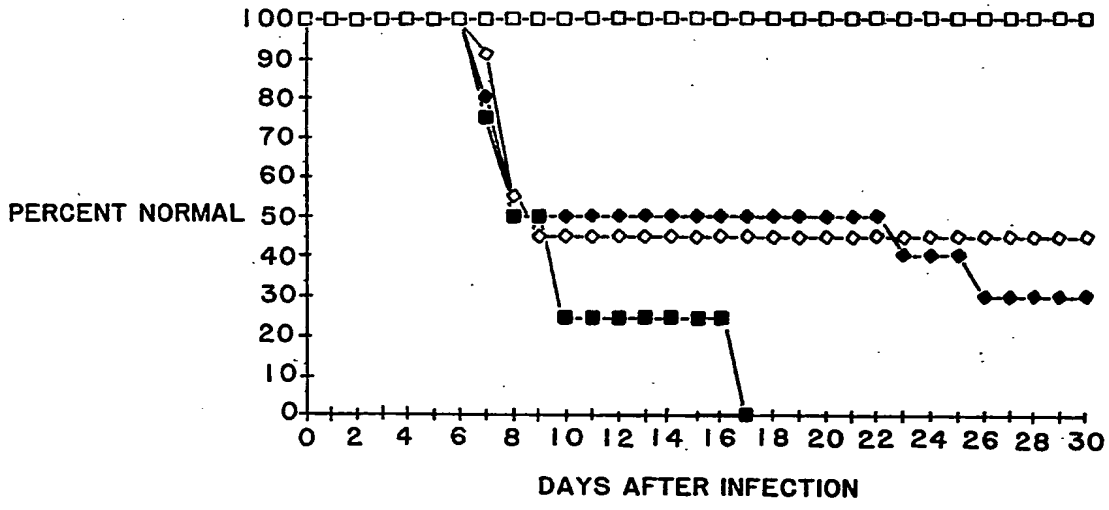


FIG. 3

0203676

FIG. 2A

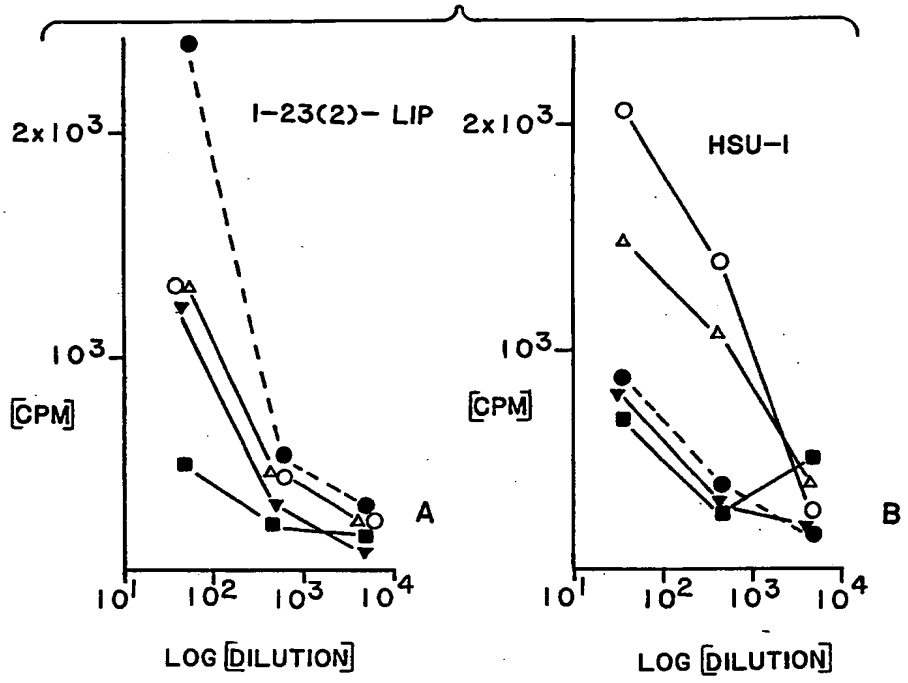


FIG. 2B